II. REMARKS

Preliminary Remarks

Claims 12, 14, and 18 are amended, and new claims 28-32 are added.

(a) Claim 12 is amended to be directed to:

A method for predicting alternative splicing transcripts in tissue samples comprising, for each tissue sample, the steps of

(i) preparing a test sample comprising labeled nucleic acid molecules having sequences that provide a match to mRNA sequences in the tissue sample;

(ii) incubating the labeled nucleic acid molecules of each test sample with an array of oligonucleotide probes having sequences of alternative splicing regions of mRNAs expressed in the tissue sample, under conditions in which hybridization of complementary nucleic acids occurs:

(iii) scanning to quantitatively detect labeled nucleic acid molecules that hybridize to the array of oligonucleotide probes;

(iv) preprocessing data resulting from the scanned hybridization reactions; and

(v) performing a first splice variant prediction to produce first splice variant prediction data.

The amendment of claim 12 replaces the steps of "performing test sample preparation and hybridization for a set of tissue samples during which hybridization reactions of the set of tissue samples are scanned" that were specified in the original claim with steps (i)-(iii) of the amended claim. Support for the amendment is found in the specification in paragraph [0019] on page 4, which provides a general description of gene expression profiling; and paragraphs [0021] and [0031]-[0034], which describe using a hybridization array such as a gene chip to collect data for expression of sequences present in alternative splicing regions.

(b) The preamble of claim 14 is amended to refer to "the steps of sample preparation, and hybridization, and scanning," to more closely correspond to the subject matter of the body of the claim.

(c) Claim 18 is amended by replacing "tasks" with "steps," for which antecedent basis is clearer, and also by specifying that "one of the steps the computer is caused to perform is the step of performing a first splice variant prediction to produce first splice variant prediction data" support for which is found, for example, in original claim 12.

(d) New claims 28-30 are directed to a computer readable medium having instructions stored thereon which, when executed, cause a computer to perform the steps of:

(i) preprocessing data resulting from scanned hybridization reactions obtained in a method for predicting alternative splicing transcripts in tissue samples; and

(ii) performing a first splice variant prediction to produce first splice variant prediction data, as described in claims 12, 14, and 15.

Examples of computer readable media that can store instructions which, when executed, cause a computer to perform steps of the claimed method are described in paragraphs [0026] and [0027] on page 7.

(e) New claims 31 and 32 are directed to a the computer readable medium of claim 28, which also has instructions stored thereon which, when executed, cause a computer to perform a second splice variant prediction to produce second splice-variant prediction data, as described in original claims 13 and 17.

Patentability Remarks

Improper restriction and withdrawal of claims 19-27

The applicants strenuously object to and traverse the imposed withdrawal of claims 19-27 on the grounds that these claims allegedly are directed to subject matter that is "distinct from the elected subject matter." The official action alleges that claims 19-27 introduce critical limitations and steps such as "a step of selecting probes based on a defined threshold" that are not required for the invention of claims 12-17. It is the applicants' position that **Claims 19-27 simply further define, in a different manner, the invention of claims 12-17**.

The first step of the method of claim 19 corresponds to the next-to-last step of claim 12 and to the subject matter of claim 15 - preprocessing the raw scan data. This part of the claimed method is described in paragraph [0041] on page 10. The second, third, and fourth steps of claim 19 correspond to the last step of claim 12 and to claim 16 - performing a first splice variant prediction, as described in paragraphs [0042] to[0047] on pages 10-12. In particular, the step of:

"combining the difference and ratio tables to generate a signal strength table"

is described in paragraph [0043].

The step of :

"creating a relative signal strength table by normalizing the signal strength table across tissues represented in the expression profiling data"

is described in paragraphs [0044] to [0046].

The step of :

"calculating final ratios indicative of the differential relative expression of the probes in the tissues represented in the expression profiling data using the relative signal strength table"

is also described in paragraph [0046] (see the top of page 12), and the step of:

"selecting probes with final ratios higher than a defined threshold"

is described in paragraph [0047].

Contrary to the examiner's allegation, selection or identification of the probes that have high final ratios ("higher than a defined threshold") is clearly described in the application as an action taken in performing a first splice variant prediction (the SPLICE phase). The first splice variant prediction identifies probes in the hybridization array that give a clear expression signal and are candidate probes that have a relatively high probability of being associated with sequences in alternative splicing regions of mRNAs that are expressed in a tissue sample of interest (see paragraph [0047]).

The final (sixth) step of claim 17:

"predicting selected probes as likely alternative splicing transcripts based on one or more factors selected from the group consisting of location on a gene and proximity to other selected probes"

corresponds to claim 13, which is directed to a further step of the method of claim 12, comprising performing a second splice variant prediction to produce second splice-variant prediction data. Performing a second splice variant prediction (the NEIGHBORHOOD phase) is described in paragraphs [0048] to [0051] on pages 12-13 of the application.

Dependent claims 20-25 are similarly directed to elements of the method for predicting alternative splicing transcripts of original claims 12 and 13, and are similarly disclosed and described in paragraphs [0043] to [0051] on pages 10-13 of the specification. Claim 26 is directed to a step of outputting a list of selected probes identified as likely alternative splicing transcripts produced by the method of claim 19, and claim 27 is directed to computer-readable medium having instructions stored thereon which, when executed, cause a computer to perform the method of claim 19. The application describes the subject matter of both of these claims as associated with the invention defined by claims 12-17. Paragraph [0027] on page 7 describes output by a user interface, and paragraph [0051] on page 13 describes production of lists of probes meeting the criteria set for identifying probes that are likely to correspond to alternative splicing transcripts. Paragraphs [0026] and [0027] on page 7 similarly describe a computer readable medium storing instructions which, when executed, cause a computer to perform steps of the claimed method.

As discussed above, all of the features or elements of withdrawn claims 19-27 are part of the invention of claims 12-17. **The applicants therefore respectfully request that claims 19-27 be re-instated and examined on their merits.**

Objections:

The official action objected to the amendment of paragraph [0033] in the response filed February 2, 2004, under 35 U.S.C. §132, because the reference to the addition of serum "to the cells to ensure a proper growth environment" in the amended paragraph is considered to be new matter. Paragraph [0033] is amended back to its original form, the meaning of which would be clear to a person of skill in the art. Withdrawal of the objection is respectfully requested.

35 U.S.C. §112, Second Paragraph

(a) Claims 12-18 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite, because the meaning of the term "uninformative probes" in claim 16 is considered to be unclear. In particular, the examiner states that it is unclear what criteria are used to determine that a probe is "uninformative;" *i.e.*, whether this is determined by lack of signal or by false signal due to non-specific hybridization.

The applicants respectfully traverse this ground for rejection. The terms "uninformative probes" and "non-informative probes" are used interchangeably in the application, and are clearly and functionally defined in the application as referring to those probes for which the difference between normalized, scaled signal strength values for the perfect match and the mismatch probe is less that a cut-off threshold. Uninformative or non-informative probes are described as being probes for which there is little or no detectable expression of a corresponding mRNA in a particular tissue sample. See lines 1-10 of paragraph [0044] on page 11. Note that according to the written description, a probe will be uninformative either if the corresponding transcript is unexpressed, or if both the mismatch and perfect match probes hybridize to the labeled sequence with about the same affinity.

(b) Claims 12-18 were further rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite because the preamble of claim 12 states that the claim is directed to '[a] method for predicting alternative splicing transcripts using DNA chip expression data," but the steps of the method of claim 12 do not refer to a DNA chip or to expression data.

Claim 12 has been amended so that references to a hybridization array and to collection and analysis of expression data are in the body of the claim rather than the preamble.

(c) Claim 18 was further rejected under 35 U.S.C. §112, second paragraph, because the term "tasks" is considered to lack sufficient antecedent basis.

Claim 18 has been amended by replacing the word "tasks" with "steps."

The applicants respectfully submit that persons of skill in the art would clearly understand the meaning and the metes and bounds of the amended claims, and withdrawal of the rejection of claims under 35 U.S.C. §112, second paragraph, is therefor respectfully requested.

35 U.S.C. §112, first paragraph

(a) Claims 14 and 18 were rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement by referring to new matter. Specifically, deletion of lines 5-7 from original claim 14, which specify purification by phenol chloroform extraction and ethanol precipitation, was alleged to be new matter because the use of these purification procedures is considered to be a required step of the claimed invention.

The applicants respectfully traverse this ground of rejection. The claimed method employs known methodology in preparing tissue samples, isolating mRNA, preparing test samples containing labeled nucleic acid molecules that provide a match to sequences of the mRNA, preparing oligonucleotide probe hybridization arrays, hybridizing the labeled nucleic acid molecules to the hybridization arrays, and scanning to quantitatively detect labeled nucleic acid molecules bound specifically to the hybridization arrays. See paragraphs [0031] to [0040] of the specification. At the time of filing, purification of nucleic acids using phenol chloroform extraction and ethanol precipitation was one of several commonly used techniques for preparing nucleic acids prior to a hybridization assay. Other methods, such as protease treatment in combination with spin column chromatography or magnetic bead isolation were also known and commonly used. The specification expressly describes using such methods to purify RNA (see line 12 paragraph [0039] on p. 9), and skilled persons would have known that similar methods were available for purifying DNA. The description of the invention does not suggest or imply that purification of DNA using phenol chloroform extraction and ethanol precipitation is a critical step of the invention. The Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement (Fed. Reg., Vol. 66, No. 4, Jan. 5, 2001) state that in order to find that omission of an element of an invention from a new or amended claim violates the "written description" requirement of 35 U.S.C. 112, first paragraph, the omitted element must be an essential or critical feature of the invention. Moreover, the statement of the rejection must accord with a thorough reading and evaluation of the application, and must present evidence or reasons why one of skill in the art would not recognize that the written description of the

invention provides support for the claim (see p. 1105). While the Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement are not rigid rules to which patent examiners must conform, they are based on applicable legal decisions and are promulgated by the U.S. Patent and Trademark Office to guide applicants in the proper preparation of patent applications. As such, the applicants submit that conformance with the requirements of the guidelines set forth therein is evidence of compliance with the "written description requirement" of 35 U.S.C. 112, first paragraph.

As discussed above, persons of skill in the art at the time of filing recognized that DNA purification by phenol chloroform extraction and ethanol precipitation was only one of several known and available methods for preparing DNA for hybridization, and would not have considered it to be an essential or critical feature of the invention. In addition, persons of skill in the art at the time of filing would reasonably have considered that claims that do not specify a particular method of purifying cDNA would be supported by the written description of the invention. Therefore, withdrawal of the rejection of claims 14 and 18 under 35 U.S.C. §112, first paragraph, for lack of written description, is respectfully requested.

(b) Claim 14 was further rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement because the step of purifying and quantifying DNA shown in Figure 3 was considered to be a required step of the claimed invention, and its omission from the description of the invention of claim 14 was considered to be new matter.

The applicants respectfully traverse this ground of rejection for reasons similar to those stated above with respect to the rejection of claims 14 and 18 under 35 U.S.C. §112, first paragraph, for lack of written description. The Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement (*Id.*) state that in determining whether the disclosure satisfies the written description requirement for the claimed subject matter, the examiner

> "should review the claims and the entire specification, including the specific embodiments, figures, and sequence listings, to understand how applicant provides

support for the various features of the claimed invention. (ref. omitted) The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention." (*Id.*)

The guidelines expressly state that, with respect to original claims,

"Possession may be shown in many ways. For example, possession may be shown, *inter alia*, by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention." (ref. omitted) (*Id.*)

With regard to assessing whether a skilled person would have regarded the applicant as being in possession of the claimed invention, the guidelines further state that,

"Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient." (*Id.*, page 1106)

The guidelines further state that

> "The absence of definitions or details for well established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, ¶ 1, for lack of adequate written description." (*Id.*, page 1105)

and

> "What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. (ref. omitted)) If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. (ref. omitted) (*Id.*, page 1106).

From the foregoing, it is clear that the claims need not be provide a mirror-like reflection of every detail of the disclosed methodology. Implication and inherence may account for details of a claimed method where one of skill in the art would recognize that the details are present, and would understand that the applicants had possession of the claimed invention at the time of filing. In the present case, the claim 14 was rejected under 35 U.S.C. §112, first paragraph, because the step of purifying and quantifying the DNA as shown in Figure 3 is considered to be a required step, and by not specifying this step, the claim fails to comply with the written description requirement of 35 U.S.C. §112, first paragraph. This reasoning does not hold up to the type of analysis called for by the guidelines as discussed above. Claim 14 includes the steps of:

> preparing double-stranded cDNA from the extracted total RNA; and
>
> performing a transcription reaction using the cDNA to produce cRNA.

Although claim 14 does not expressly specify purifying and quantifying the DNA, one of skill in the art would reasonably expect that the step of preparing the double-stranded cDNA prior to using it as a substrate in a transcription reaction would include the steps of purification and quantification. Accordingly, one of skill in the art would regard the applicants as having been in possession of the claimed invention. Withdrawal of the rejection of claim 14 under 35 U.S.C. §112, first paragraph, for lack of written description, is therefore respectfully requested.

(c) Claim 18 was further rejected under 35 U.S.C. §112, first paragraph, because Figures 2-6 are considered to describe a system and method that comprises more than two

steps, so that claim 18, which is directed to a computer readable medium having instructions stored thereon which, when executed, cause a computer to perform only "one or more tasks of the method of claim 12" is alleged to constitute new matter. The applicants respectfully traverse this ground of rejection. Persons of skill in the art at the time of filing recognized that some of the steps of claim 12, such as test sample preparation, hybridization, and scanning, could be performed by hand, or could be automated and placed under computer control. Therefore, one of skill in the art would not reasonably consider that computer control of all of the steps of claim 12 is a required feature of the claimed invention. Accordingly, withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

35 U.S.C. §103(a)

Claims 12-18 were rejected under 35 U.S.C. §103(a) as allegedly being obvious in view of Emmert-Buck et al. ("Emmert-Buck") in view of Schena et al. ("Schena"). The applicants respectfully traverse the rejection of the claims as having been obvious in view of Emmert-Buck in combination with Schena, as neither of the cited references, alone or in combination, would have described or suggested the claimed invention to a person of ordinary skill in the art.

Emmert-Buck reviewed the state of the art of molecular profiling, which is described as the identification of genes or gene combinations that mediate aspects of cellular physiology. In arguing that the claimed invention would have been obvious, the official action pointed to a general discussion of molecular profiling on pages 1109-1110 which described advances in methods for analyzing tissue-specific patterns of gene expression and the development of computer modeling algorithms global gene expression that are expected to lead to a better understanding of disease processes. The official action also pointed to page 1112 of Emmert-Buck, which described the discovery of a gene that is specifically up-regulated in prostate tumor cells that was achieved using a gene chip to analyze differential gene expression. The method used by Emmert-Buck is one in which sequences of transcripts from normal and tumor cells are amplified, hybridized to an oligonucleotide micro-array and detected, and transcript sequences that are differentially expressed in tumor cells relative to normal cells are identified. Emmert-Buck also described the gene that was found to be up-

regulated specifically in tumor cells as a gene that encodes a splice variant transcript. In the description of this research, Emmert-Buck cited two references. (1) Chuaqui et al. (Urology. 1997 Aug; 50(2):302-7, abstract enclosed) described the method of using a gene chip to analyze differential gene expression in normal and cancerous human prostate tissue and the discovery of a gene that is expressed at higher levels in prostate tumor cells than in normal cells. (2) Cole et al. (Genomics. 1998 Jul 15; 51(2):282-7, abstract enclosed) described sequencing a cDNA of the differentially expressed gene and reported that the gene encodes a splice variant transcript.

The differential expression profiling methods described by Emmert-Buck would permit one to identify a gene that is up- or down-regulated in one cell type relative to its expression in another, but Emmert-Buck neither described nor suggested the analytical of the claimed invention, which comprises performing a splice variant prediction to produce splice variant prediction data, which enables one to predict if a gene is expressed as alternative splice variants in two or more different tissue types.

This deficiency in Emmert-Buck, the primary reference, is not remedied by Schena, which describes sensitive high-throughput methods for global gene expression analysis in which gene chips are used to detect alterations in gene expression in response to stimuli. Like Emmert-Buck, Schena expresses enthusiasm that coming technological advances will greatly contribute to our functional understanding of the human genome. However, like Emmert-Buck, Schena also did not describe or suggest the claimed invention comprising performing a splice variant prediction to produce splice variant prediction data, which enables one to predict if a gene is expressed as alternative splice variants in two or more different tissue types. The present invention obviates the need to perform DNA sequencing to determine if a differentially expressed transcript of interest is a splice variants as described by Emmert-Buck (see the enclosed Cole et al. abstract). Given that the prior art neither described nor suggested the claimed invention, withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

Conclusion

All rejections having been addressed, it is respectfully submitted that the present application is in condition for allowance and a Notice to that effect is earnestly solicited. If any points remain in issue, which the examiner feels may be best resolved through a personal or telephone interview, he is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,

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Attachments:

Copies of

(1) Chuaqui et al., Urology. 1997 Aug; 50(2):302-7 (abstract only)

(2) Cole et al., Genomics. 1998 Jul 15; 51(2):282-7 (abstract only)

and a Form PTO-1449 citing the same.

Urology. 1997 Aug;50(2):302-7.

Identification of a novel transcript up-regulated in a clinically aggressive prostate carcinoma.

Chuaqui RF, Englert CR, Strup SE, Vocke CD, Zhuang Z, Duray PH, Bostwick DG, Linehan WM, Liotta LA, Emmert-Buck MR.

Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland, USA.

OBJECTIVES: To identify differentially expressed genes in tumor cells of patients with prostate cancer by means of tissue microdissection and targeted differential display. METHODS: RNA was recovered from pure populations of microdissected normal epithelium and invasive tumor from frozen tissue sections of a radical prostatectomy specimen. Reverse transcription-polymerase chain reaction (PCR) using arbitrary and zinc finger PCR primers was performed. RESULTS: A 130-base pair product was identified that appeared selectively in the tumor sample. DNA sequence analysis revealed it to be a clone from the expressed sequence tag database (GenBank accession R00504). Microdissection of normal epithelium and the corresponding invasive tumor was subsequently performed on a test panel of 10 prostate carcinoma specimens. Comparison of R00504 levels in normal epithelium and invasive carcinoma, using beta-actin as an internal control, showed the transcript to be substantially overexpressed in 5 of 10 carcinomas. Northern blotting revealed R00504 to be a 2.6-kilobase gene. CONCLUSIONS: A novel transcript up-regulated in an aggressive prostate carcinoma was identified using degenerate zinc finger primers in microdissected tissue samples. The approach used in this study may be helpful in quantitative comparison of known genes and identification of novel genes in microdissected human tissue samples.

PMID: 9255310 [PubMed - indexed for MEDLINE]

Genomics. 1998 Jul 15;51(2):282-7.

Related Articles, Links

cDNA sequencing and analysis of POV1 (PB39): a novel gene up-regulated in prostate cancer.

Cole KA, Chuaqui RF, Katz K, Pack S, Zhuang Z, Cole CE, Lyne JC, Linehan WM, Liotta LA, Emmert-Buck MR.

Laboratory of Pathology, Division of Clinical Sciences, National Cancer Institute, National Institues of Health, Bethesda, Maryland 20892, USA.

We recently identified a novel gene (PB39) (HGMW-approved symbol POV1) whose expression is up-regulated in human prostate cancer using tissue microdissection-based differential display analysis. In the present study we report the full-length sequencing of PB39 cDNA, genomic localization of the PB39 gene, and genomic sequence of the mouse homologue. The full-length human cDNA is 2317 nucleotides in length and contains an open reading frame of 559 amino acids which does not show homology with any reported human genes. The N-terminus contains charged amino acids and a helical loop pattern suggestive of an srp leader sequence for a secreted protein. Fluorescence in situ hybridization using PB39 cDNA as probe mapped the gene to chromosome 11p11.1-p11.2. Comparison of PB39 cDNA sequence with murine sequence available in the public database identified a region of previously sequenced mouse genomic DNA showing 67% amino acid sequence homology with human PB39. Based on alignment and comparison to the human cDNA the mouse genomic sequence suggests there are at least 14 exons in the mouse gene spread over approximately 100 kb of genomic sequence. Further analysis of PB39 expression in human tissues shows the presence of a unique splice variant mRNA that appears to be primarily associated with fetal tissues and tumors. Interestingly, the unique splice variant appears in prostatic intraepithelial neoplasia, a microscopic precursor lesion of prostate cancer. The current data support the hypothesis that PB39 plays a role in the development of human prostate cancer and will be useful in the analysis of the gene product in further human and murine studies.

PMID: 9722952 [PubMed - indexed for MEDLINE]